THE OCCURRENCE AND COMPOSITION OF THE SUGARS IN THE HONEYDEW OF ERIOCOCCUS CORIACEUS (MASK.)

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Synopsis

The honeydew secreted by the Blue Gum Scale, *Eriococcus coriaceus* (Mask) contains, inter alia, seven sugars. One of these has not been previously reported, two have not been found previously occurring in nature and one is a relatively rare sugar. These sugars all have the unusual (1→3) glycoside linkage between portions of the molecule. The sugars and the method of their identification are described.

Eriococcus coriaceus (Mask.) is a scale insect which lives almost exclusively on eucalypts. Tillyard (1926) states that nearly thirty species of *Eriococcus* are known in Australia. The commonest is *Eriococcus coriaceus* (Mask.) which infests the branches of gum trees. It does little harm in Australia but when accidentally introduced into New Zealand it killed off many thousands of acres of blue gum (*Eucalyptus globulus*) until controlled by the introduced Australian ladybird beetle *Rhizobius ventralis* Er.

Froggatt (1921) says " This is the commonest species of the genus, and it is widely distributed over Australia upon many different species of *eucalyptus*. There are many variations in colour but the general form is regular unless massed together. We always find it more vigorous upon all kinds of cultivated gums in gardens and avenues, than in the native bush, where it has many enemies among the ladybird beetles and scale-eating moths ".

Hoy (1963) reports it as occurring on *E. amygdalina*, *E. cladocalyx*, *E. coccifera*, *E. globulus*, *E. gunnii*, *E. regnans*, and *E. stuartiana*. The material used in this investigation was obtained from *E. maculata* and *E. punctata*. No other species of *Eucalyptus* in this, the Hunter River district, appeared to be attacked.

The insect was first described by Maskell in 1893 but an apparently more reliable description is given by Froggatt (1921).

Eriococcus secretes a honeydew which is much sought by ants. In many cases the trail of ants up a tree and along a branch leads to the discovery of a colony of scale. In some cases the secretion of honeydew is so copious that all the leaves and most of the twigs below the colony are thickly covered with the honey. The material used in this investigation was obtained by washing it from the leaves and twigs, filtering and then concentrating the solution under reduced pressure. The dilute solution had a pH 5·8. This acidity was considered to be too low to induce any appreciable hydrolysis during concentration but as a further safeguard the solution was concentrated under reduced pressure. A second sample of honeydew extracted and evaporated under atmospheric pressure showed no significant difference in composition from the first sample. This indicated the precaution was an additional safeguard against hydrolysis. The concentrated honeydew had the colour and consistency of treacle and when fully dehydrated under vacuum looked like dark toffee.

A complete analysis of the dehydrated material was not made, as only the sugars in the honeydew formed the subject of this investigation. However, Table 1 gives the results of some analyses that were made.

The secretion of *Eriococcus coriaceus* is of considerable interest in that it contains a sugar which has not been previously described, two sugars which have been known previously only as prepared in the laboratory and have not been reported as occurring naturally, and as a third feature of interest, the above mentioned sugars together with a rare one which occurs naturally, all contain only glucose and fructose units joined by one or more (1→3) glycosidic linkages.

Table 1

Ash..	11·4
Total sugars (as glucose) ..	33·15
Protein	2·6
Esters and acids	5·0
Not determined	47·85

Analysis of Ash

Na_2O	25·76
K_2O	12·12
CaO	6·95
MgO	9·87
SiO_2	2·86
P_2O_5	13·36
SO_4	21·76
Fe, Al, Cl, CO_2	not determined
	92·68

An examination of the mixed sugars by paper chromatography revealed the presence of seven. These have been identified as eriose, raffinose, melezitose, laminaribiose, turanose, glucose and fructose.

The first named sugar has not, as far as I am aware, been reported before and the name eriose is proposed for it. Laminaribiose and turanose have not previously been reported as occurring in natural products, they having been prepared in the laboratory by the hydrolysis of laminarin and melezitose respectively.

The method of identification of the sugars is detailed below.

The only sugar of the seven shown to be present, which has not a character common with the rest is raffinose. An explanation of the reason for its presence is offered below.

The names of the sugars, their systematic names and some of their chromatographic data are given in Table 2.

Table 2

Sugar	Systematic name	R_G *	Colour of stain †
Eriose	O-D-glucopyranosyl (1→3)-O-D fructo furanosyl (2→1) O-D-glucopyranosyl (3→1) O-D-glucopyranoside	0·19	Victorian mauve C.C.297
Raffinose	O-D-galactopyranosyl (1→6) O-D-glucopyranosyl (1→2) O-D-fructofuranoside	0·24	Olive drab C.C.362
Melezitose	O-D-glucopyranosyl (1→3) O-D-fructofuranosyl (2→1) O-D-glucopyranose	0·36	Victorian mauve C.C.297
Laminaribiose	O-D-glucopyranosyl (1→3) O-D-glucopyranose	0·54	Lavender mauve C.C.296
Turanose	O-D-glucopyranosyl (1→3) O-D- fructofuranoside	0·64	Verbena mauve C.C.157
Glucose	D-Glucopyranose	1·0	Lavender grey C.C.343
Fructose	D-fructofuranose	1·20	Apricot buff C.C.44

* The R_G was determined on Whatmans No. 1 paper using Butanol:acetone:water 3:4:1 as eluent. The paper was dipped in aniline-diphenylamine phosphate solution in acetone.

† The colours and colour code refer to the British Colour Council Dictionary of Colours.

The presence of raffinose amongst the sugars secreted by *Eriococcus* seems anomalous, but it might be explained as having been sucked up in the sap by the insect which is unable to assimilate it and it is then excreted unchanged together with the other sugars. It has been shown Basden (1965) that the phloem sap of eucalypts contains from 5% to 10% raffinose together with about 80% sucrose and 10% glucose and fructose. Raffinose is present as about 10% of the honeydew so that this assumption is not improbable. No galactose nor galactoside other than raffinose is present among the sugars. If the raffinose absorbed by the insect were hydrolysed in the digestive tract one would expect it to reappear in the secretion in some other form as is the case in the secretion of *Eurymela distincta* (Signoret) (Basden 1966).

The remaining sugars secreted by the insect are composed of glucose and fructose molecules united in various combinations to yield those listed in Table 2.

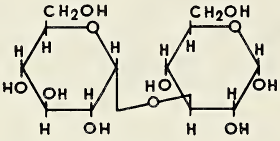

Fig. 1

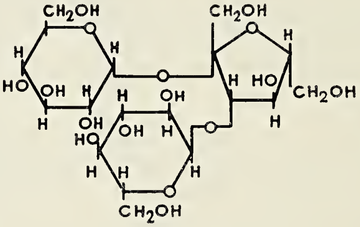

Fig. 3

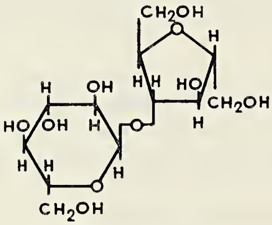

Fig 2

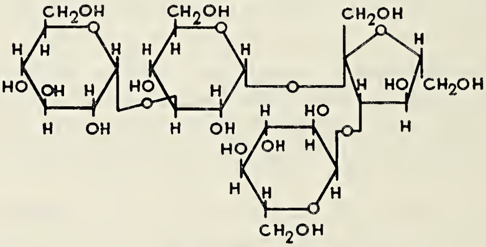

Fig. 4

Eriose is a tetrasaccharide which, as far as the author is aware, has not been previously identified nor described. It comprises about 20% of the sugars of the honeydew. Not sufficient of the sugar has yet been obtained to permit its physical properties to be examined but it has been extensively examined chemically and its formula determined. It is O-α-D-glucopyranosyl (1→3) fructofuranosyl (2→1) O-α-D-glucopyranosyl-(3→1)-O-D-glucopyranoside. Its structure is shown in Fig. 4. It will be observed that its molecule consists of a laminaribiose moiety Fig. 1 joined to a turanose molecule Fig. 2 by a (1→2) linkage as in sucrose. Also it will be seen from the formula that if one of the glucose molecules of the laminaribiose portion is removed the remainder is melezitose Fig. 3, and if the second molecule of glucose is removed the remainder is turanose. Thus these four sugars belong as it were to the same family. The weakest link between the four portions of the molecule is the (1→2) linkage in the middle and is the point of cleavage of the molecule during hydrolysis. It is because this bond is so vulnerable that it has not been possible to obtain melezitose as one of the products.

The position of the spot on the chromatogram of eriose is consistent with its being a tetrasaccharide. Further experimental work is now being done in an effort to synthesize this new sugar.

Melezitose was first described by Morninglane, Duponchel and Bonastre (1822) during the examination of a secretion from the larch tree (*Larix europaea* DC.). They called the secretion Manne de Briançon. Many years later interest in this manna was revived by Alekhine (1889). He hydrolysed the sugar obtained by recrystallising the manna from glacial acetic acid. By using 1% sulphuric acid and heating on a steam bath he found that its molecular rotation changed from +87·5° to +64° in half an hour. It remained nearly constant during the next half hour, but then the rotation fell slowly and became constant at about +50°. The first stage resulted in the formation of a monosaccharide and a disaccharide. The former was shown to be D-glucose and the latter a new sugar which he named turanose from Turan, the old Persian name of the general region north-east of Persia from which the manna had come. Four years after Alekhine's paper was published, Maquenne (1893) reported the presence of melezitose in honeydew from *Tilia.* In later years other writers have reported its occurrence in poplar (*Populus nigra* L.) in *Laburnum anagyroides* Griseb. and *Pinus virginiana* Mill.

This sugar is now obtained from the manna of Douglas Fir (*Pseudotsuga menziesii* (Mirb.) Franco) and in larger quantities from " melezitose honey " in which the sugar has been gathered by bees and incorporated in the honey. In some seasons it constitutes 10% to nearly 20% of the honey. Hudson (1946) states " The melezitose of the honey is not made by the bees ; the trisaccharide was found in honeydew that was taken from pine branches." Arnhart (1926 and 1930) stated that the saccharine material of honeydew is to be attributed to the activity of plant lice of several kinds.

The formula for melezitose was established by Georges Tanret (1906) as $C_{18} H_{32} O_{16} 2H_2O$ (Fig. 3).

In the honeydew of *Eriococcus coriaceus*, melezitose constitutes about 15% of the sugars present. It was identified by hydrolysing a specimen with 1% sulphuric acid at 100° for 30 minutes and then chromatographing the resultant sugars. These were glucose and turanose in equal quantities. The sugar was further identified with melezitose by subjecting it to a two dimensional chromatogram using an authentic specimen of melezitose as a control. The R_G and the colour derived from a spray reagent were identical with those of the control.

Laminaribiose has not, so far, been reported as occurring in nature. It was obtained (Barry 1941) as one of the products of the partial hydrolysis of laminarin, a reserve polysaccharide of some brown seaweeds. Barry used the enzymes of the digestive juice of the snail *Helix pomata*, to achieve the hydrolysis. He determined the structure of laminaribiose to be as shown in Table 2 and Fig. 1. In eriococcus honeydew it is present as about 10% of the total sugars. It has been identified by its yielding only glucose on hydrolysis with normal sulphuric acid and also by the presence of the (1→3) linkage as indicated by the mauve colour of its chromatogram when sprayed with aniline diphenylamine phosphate reagent. Its chromatographic behaviour places it among the disaccharide group of sugars.

The disaccharide turanose constitutes about 10% of eriococcus honeydew. It has not been reported as occurring in nature. It was first prepared by Alekhine (1889) by the partial hydrolysis of melezitose from larch manna. Five years later Emil Fischer (1894) prepared turanose phenylosazone and reported many of the properties of the osazone and of the sugar. However another fifty years elapsed before the structure of the sugar was proved. (Hudson 1944 and 1950).

Turanose was identified in eriococcus honeydew by its yielding equal amounts of glucose and fructose when hydrolysed with normal sulphuric acid at 100° for thirty minutes. Its phenylosazone was identical with that of an authentic sample of turanose and a two dimensional chromatogram was also identical with that of turanose.

Acknowledgement

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